DOCKET NO.: ORT-1508/ JJPR-0021 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: **Fredrick Kamme and Jessica Y. Zhu**	Confirmation No.: **9944**
Serial No.: **10/080,795**	Group Art Unit: **1637**
Filing Date: **February 22, 2002**	Examiner: **Young J. Kim**

For: **Method for Generating Amplified RNA**

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Commissioner for Patents
P.O. Box 1450
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Sir:

APPELLANT'S BRIEF PURSUANT TO 37 C.F.R. § 41.37

This brief is being filed in support of Appellant's appeal from the final rejection of claims 1, 2, 4-14 and 16-23 dated July 27, 2006. A Notice of Appeal was filed on September 27, 2006.

1. REAL PARTY IN INTEREST

The real party in interest in the above-identified patent application is Ortho-McNeil Pharmaceutical, Inc., a corporation of New Jersey, which is the assignee of Fredrik Kamme and Jessica Y. Zhu, as reflected in an assignment recorded in the USPTO on August 26, 2003, at Reel 013912, Frame 0217.

2. RELATED APPEALS AND INTERFERENCES

There are no other appeals or interferences known to Appellant, Appellant's legal representative, or the assignee that will directly affect or be directly affected by or have a bearing on the Board's decision in the present Appeal.

3. STATUS OF CLAIMS

Claims 1, 2, 4-14 and 16-23 are pending in this patent application and are the subject of this appeal. A listing of the claims is provided in Appendix A.

4. STATUS OF AMENDMENTS

No amendment was filed subsequent to the final Office Action dated July 27, 2006.

5. SUMMARY OF CLAIMED SUBJECT MATTER

The claimed invention relates to certain processes for amplifying RNA from cells.

More particularly, in one general aspect, the invention is directed to a method of amplifying at least one mRNA in a sample containing a plurality of different mRNAs, (see, *e.g.*, specification at page 5, lines 11-12) comprising the following steps:

a) synthesizing first strand cDNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in said sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in the mRNA so as to prime synthesis in a direction toward the 5' end of the mRNA; (see, *e.g.*, specification at page 5, lines 13-19.)

b) synthesizing a second strand of cDNA by contacting under conditions conducive to a thermostable DNA polymerase activity, said conditions comprising an incubation temperature of from 45°C to 80°C, the first strand cDNA with (i) a thermostable DNA polymerase, which is Bst DNA polymerase large fragment, and (ii) a thermostable RNase H, thereby forming double stranded cDNA; and (see, *e.g.*, specification at page 5, lines 3-4 and lines 20-24; and page 13, line 24 to page 14, line 3.)

c) transcribing the double stranded cDNA into cRNA by contacting the double stranded cDNA with an RNA polymerase under conditions conducive to RNA polymerase activity, such that cRNA is produced. (see, *e.g.*, specification at page 5, lines 25-28.)

In another general aspect, the invention relates to a method for comparing the presence or amount of at least one mRNA of interest in a first sample and in a second sample, the first sample and the second sample each containing a plurality of different mRNAs from one or more cells. (see, *e.g.*, specification at page 6, lines 12-15.) The method comprises the steps of:

a) synthesizing first strand cDNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in said first sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in the mRNA so as to prime synthesis in a direction toward the 5' end of the mRNA; (see, *e.g.*, specification at page 6, lines 12-19.)

b) synthesizing a second strand of cDNA by contacting, under conditions conducive to thermostable DNA polymerase activity, said conditions comprising an incubation temperature of from 45°C to 80°C, the first strand cDNA with (i) a thermostable DNA polymerase, which is Bst DNA polymerase large fragment, and (ii) a thermostable RNase H, thereby forming double stranded cDNA; (see, *e.g.*, specification at page 6, lines 19-22; and page 13, line 24 to page 14, line 3.)

c) transcribing the double stranded cDNA into cRNA by contacting the double stranded cDNA with an RNA polymerase under conditions conducive to RNA polymerase activity, such that cRNA is produced; (see, *e.g.*, specification at page 6, lines 22-25.)

d) labeling the cRNA produced in step (c) with a first label; (see, *e.g.*, specification at page 6, line 25.)

e) repeating steps (a) – (c) with said second sample; (see, *e.g.*, specification at page 6, lines 25-26.)

f) labeling the cRNA produced in step (e) with a second label distinguishable from said first label; (see, *e.g.*, specification at page 6, lines 26-27.)

g) detecting or measuring the mRNA of interest in the first sample by contacting the cRNA labeled with said first label with a polynucleotide capable of hybridizing to said cRNA of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide and said cRNA; (see, *e.g.*, specification at page 6, line 27 to page 7, line 3.)

h) detecting or measuring the mRNA of interest in the second sample by contacting the cRNA labeled with said second label with said polynucleotide capable of hybridizing to said cRNA of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide and said cRNA; and(see, *e.g.*, specification at page 7, lines 3-7.)

i) comparing the mRNA of interest detected or measured in said first sample with the mRNA of interest detected or measured in said second sample. (see, *e.g.*, specification at page 7, lines 7-8.)

6. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Whether the subject matter of claims 1, 2, 4-14 and 16-23 is patentable under 35 U.S.C. §103(a) over U.S. patent 6,566,502 B1 to Mack et al. ("Mack") in view of U.S. patent 6,406,891 B1 to Legerski et al. ("Legerski").

7. ARGUMENT

The invention as defined in claims 1, 2, 4-14 and 16-23 is patentable under 35 U.S.C. §103(a) over Mack in view of Legerski because the references, singly or combined, do not teach or suggest the claimed invention as a whole.

To establish a *prima facie* case of obviousness under 35 U.S.C. §103, it is incumbent upon the Examiner to provide a reason why one of ordinary skill in the art would have been motivated to modify a prior art reference or to combine reference teachings to arrive at the claimed invention. *Ex parte Clapp*, 227 U.S.P.Q. 972 (Bd. Pat. App. Int. 1985). The requisite motivation must stem from some teaching, or suggestion in, or inference drawn from the prior art, or from the knowledge generally available to one of ordinary skill in the art rather than from Applicants' disclosure. See for example, *Uniroyal Inc. v. Rudkin-Wiley Corp.*, 5 U.S.P.Q.2d 1434 (Fed. Cir. 1988); and *Ex parte Nesbit*, 25 U.S.P.Q.2d 1817, 1819 (Bd. Pat. App. Int. 1992). For the reasons discussed below, a proper *prima facie* case of obviousness has not been set forth.

In the final Office Action, the Examiner alleges that all of the steps recited in the instant claims are disclosed by Mack, excepting for the claim requirement that the polymerase used for the second strand cDNA synthesis is an *E. coli* DNA polymerase. The Examiner indicates that the currently claimed method differs from the teachings of Mack in employing conditions suitable for a Bst large fragment DNA polymerase rather than an *E. coli* DNA polymerase in the second strand synthesis. The Examiner then argues that Legerski improves the method of generating double stranded cDNAs, "wherein the artisan specifically recognizes the problem associated with generating a second strand cDNA synthesis from a first strand cDNA, that is, the formation of secondary structure." The Examiner goes on to state that "Legerski specifically overcomes this problem by use of a thermostable DNA polymerase *in the second strand cDNA synthesis step*, specifically contemplating Bst DNA polymerase large fragment, allowing for the synthesis of long cDNA molecules." (emphasis added).

As the disclosure of the secondary reference would have been interpreted by artisans without the benefit of hindsight knowledge of the present invention, however, Legerski does not teach *any* change to the second strand cDNA synthesis step. Rather, Legerski is concerned with problems associated with producing long cDNAs, *i.e.*, in the range of more than 5-6 kb (Legerski, Col. 6, lines 2-4). Legerski points out that the problem is caused by "the occurrence of secondary structures in the mRNA" (Legerski, Col., 6, lines 4-5) as opposed to any feature of the first strand of cDNA after it is formed. Legerski goes on to teach: "[T]hese secondary structures inhibit the progression of the reverse transcriptases and thus prevent the synthesis of full-length *first strand* cDNA" (Legerski, Col., 6, lines 5-8, emphasis added).

Indeed, Legerski describes the following method, focused on first strand cDNA synthesis (Legerski, Col. 6, lines 9-16, emphasis added):

> As described more completely below, the present invention provides a method of producing full length *first strand* synthesis of cDNA. More particularly, the invention describes a method in which cycling back and forth between a processive RT and a thermostable RT enzyme *during first strand synthesis* allows for the complete production of the *first strand* of a full length cDNA. This invention exploits the notion that the mRNA secondary structures may be removed by elevating the temperature of the RT reaction. It is possible to carry out an RT reaction at the elevated temperatures using a thermostable RT enzymes, however, although these enzymes are operative at high temperatures (55-90°) the reaction is very slow. This inefficiency can be circumvented by adding fresh processive RT enzyme once the impediment of the secondary structure has been bypassed. Thus, the synthesis of the *first strand* can be continued at the lower temperature. This cycling allows the alternate synthesis of the long chain at the lower temperature and removal of the secondary structures at the higher temperature.

It is clear that Legerski's focus is on improving the process of making full length cDNA from long mRNA by temperature cycling in first strand synthesis to remove mRNA secondary structure during that step.

With respect to second strand synthesis, Legerski teaches that Bst DNA polymerase large fragment can be used. However, Bst1 DNA polymerase is offered among a laundry list of DNA polymerases, some of which are thermostable and some of which are not (see Legerski, Col. 11, line 48 through Col. 15, line 67). The Examiner fails to point to any motivation for selectively choosing Bst1 DNA polymerase from the various DNA polymerases disclosed in the prior art. Indeed, Legerski does not even recommend Bst DNA

polymerase for thermal cycling in first strand synthesis, and even goes so far as to note that "Bst DNA polymerase cannot be used for thermal cycle sequencing" (Legerski, Col. 12, lines 11-12). Contrary to the Examiner's assertion in the final Office Action, Legerski does not distinguish between Bst DNA polymerase and Bst DNA polymerase large fragment, so that the statement that Bst DNA polymerase cannot be used for thermal cycle sequencing applies to Bst DNA polymerase large fragment. Furthermore, neither Legerski nor Mack teaches or suggests utilizing a thermostable RNAse H in the second strand synthesis reaction. Thus, Legerski and Mack nowhere teach or suggest that second strand synthesis should or could be carried out at elevated temperature, or in the presence of thermostable RNAse H, as is recited in the instant claims.

It can be seen from the foregoing that neither of the cited references provides any hint or suggestion that would have motivated the ordinarily skilled artisan to combine their teachings so as to arrive at the claimed methods. The prior art fails to teach or suggest a method where *second strand* synthesis is carried out at an elevated temperature of from 45 to 80 °C using the Bst DNA polymerase large fragment and a thermostable RNAse H. Nor does the prior art teach or suggest advantages flowing from the claimed methods, such as the ability to generate relatively large amounts of RNA from a small starting number of cells with high efficiency and in a substantially reduced time period compared to known methods for performing RNA amplification. Any combination in the absence of a "specific hint or suggestion in a particular reference" is thus necessarily the result of impermissible hindsight and is not a proper basis for a *prima facie* of obviousness. *In re Sang Su Lee,* 277 F.3d 1338 (Fed. Cir. 2002).

In sum, the combined teachings of Mack and Legerski fail to render the claimed invention as a whole obvious. Accordingly, the rejection of claims 1, 2, 4-14 and 16-23 under 35 U.S.C. § 103(a) on the basis of Mack and Legerski is in error, and should be reversed.

8. CONCLUSION

Having shown the final rejection of the pending claims under 35 U.S.C. § 103(a) as being improper, Appellants respectfully request that such decision be reversed.

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CLAIMS APPENDIX

The following constitutes a clean copy of the claims on appeal.

1. A method for amplifying at least one mRNA in a sample containing a plurality of different mRNAs comprising:
   a) synthesizing first strand cDNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in said sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in the mRNA so as to prime synthesis in a direction toward the 5' end of the mRNA;
   b) synthesizing a second strand of cDNA by contacting under conditions conducive to a thermostable DNA polymerase activity, said conditions comprising an incubation temperature of from 45°C to 80°C, the first strand cDNA with (i) a thermostable DNA polymerase, which is Bst DNA polymerase large fragment, and (ii) a thermostable RNase H, thereby forming double stranded cDNA; and
   c) transcribing the double stranded cDNA into cRNA by contacting the double stranded cDNA with an RNA polymerase under conditions conducive to RNA polymerase activity, such that cRNA is produced.

2. The method of claim 1 wherein said incubation temperature is from 55°C to 70°C.

4. The method of claim 1 wherein the conditions for synthesizing the second strand of cDNA further comprise an incubation time of from one to sixty minutes.

5. The method of claim 1, which further comprises labeling the transcribed cRNA with a fluorescent, radioactive, enzymatic, hapten, biotin, or digoxigenin label.

6. The method of claim 1, wherein the thermostable Bst DNA polymerase large fragment is present in a concentration of from 0.012 to 1.3 units/μl and the thermostable RNase H is present in a concentration of from 0.0031 to 1.3 units/μl.

7. The method of claim 5, wherein the label is fluorescent.

8. The method of claim 7 wherein the fluorescent label is fluorescein isothiocyanate, lissamine, Cy3, Cy5, or rhodamine 110.

9. The method of claim 7, wherein a first aliquot of the cRNA is labeled with a first fluorophore having a first emission spectrum, and a second aliquot of the cRNA is labeled with a second fluorophore with a second emission spectrum differing from that of the first emission spectrum.

10. The method of claim 9, wherein the first fluorophore is Cy3 and the second fluorophore is Cy5.

11. The method of claim 1 further comprising, after the transcribing step, determining the presence or absence of a pre-selected target mRNA in said sample.

12. The method of claim 1, wherein the conditions for synthesizing the second strand of cDNA further comprise an incubation time of from five to thirty minutes.

13. The method of claim 1, wherein the mRNA is extracted from at least one cell of interest, and further comprising contacting the cRNA produced in step (c) with an array containing one or more species of polynucleotide positioned at pre-selected sites on the array, under conditions conducive to hybridization; and detecting any hybridization that occurs between said one or more species of polynucleotide and said cRNA.

14. A method for comparing the presence or amount of at least one mRNA of interest in a first sample and in a second sample, said first sample and said second sample each containing a plurality of different mRNAs from one or more cells, comprising:

a) synthesizing first strand cDNA by contacting under conditions conducive to reverse transcriptase activity at least one mRNA in said first sample with (i) reverse transcriptase, and (ii) a first primer that is sufficiently complementary to a sequence in the mRNA so as to prime synthesis in a direction toward the 5' end of the mRNA;

b) synthesizing a second strand of cDNA by contacting, under conditions conducive to thermostable DNA polymerase activity, said conditions comprising an incubation temperature of from 45°C to 80°C, the first strand cDNA with (i) a thermostable DNA polymerase, which is Bst DNA polymerase large fragment, and (ii) a thermostable RNase H, thereby forming double stranded cDNA;

c) transcribing the double stranded cDNA into cRNA by contacting the double stranded cDNA with an RNA polymerase under conditions conducive to RNA polymerase activity, such that cRNA is produced;

d) labeling the cRNA produced in step (c) with a first label;

e) repeating steps (a) – (c) with said second sample;

f) labeling the cRNA produced in step (e) with a second label distinguishable from said first label;

g) detecting or measuring the mRNA of interest in the first sample by contacting the cRNA labeled with said first label with a polynucleotide capable of hybridizing to said cRNA of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide and said cRNA;

h) detecting or measuring the mRNA of interest in the second sample by contacting the cRNA labeled with said second label with said polynucleotide capable of hybridizing to said cRNA of the mRNA of interest under conditions conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide and said cRNA; and

i) comparing the mRNA of interest detected or measured in said first sample with the mRNA of interest detected or measured in said second sample.

16. The method of claim 14 wherein said sample contains total RNA or total mRNA from mammalian cells.

17. The method of claim 14 wherein the incubation temperature is from 55°C to 70°C.

18. The method of claim 14 wherein said first label is Cy3 and said second label is Cy5.

19. The method of claim 14 wherein said detecting or measuring steps (g) and (h) are carried out by a method comprising contacting said cRNA with an array containing one or more species of polynucleotide positioned at pre-selected sites on the array, under conditions conducive to hybridization; and detecting any hybridization that occurs between said polynucleotide and said cRNA.

20. The method of claim 19 wherein the array comprises a support with at least one surface and more than one different polynucleotides, each different polynucleotide comprising a different nucleotide sequence and being attached to the surface of the support in a different, selected location on said surface.

21. The method of claim 19 wherein the array has at least 1,000 polynucleotide probes per square centimeter.

22. The method of claim 14 wherein in steps (g) and (h), the steps of contacting the cRNA labeled with said first label with said polynucleotide probe, and contacting the cRNA labeled with said second label with said polynucleotide probe, are carried out concurrently.

23. The method of claim 14 wherein said first sample contains mRNAs from cells that are pathologically aberrant and wherein said second sample contains mRNAs from cells that are not pathologically aberrant.

EVIDENCE APPENDIX

None

RELATED PROCEEDINGS APPENDIX

None